

## Experimental infection of weaned piglets with enterotoxigenic *Escherichia coli* O149:F4

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### Abstract

Enterotoxigenic *Escherichia coli* is an important enteric pathogen causing post-weaning diarrhoea in piglets. Enterotoxins of *E. coli* induce the release of fluid into the intestines without apparent inflammation. Some serotypes of *E. coli*, especially O149:F4 can often be identified in association with haemorrhagic gastroenteritis. In this study we infected the weaned piglets (n = 53) by oral administration of enterotoxigenic *E. coli* O149:F4 strains isolated from piglets suffering from haemorrhagic gastroenteritis. The clinical course of infection and shedding of the challenge *E. coli* strain in the faeces of infected piglets was monitored for 14 days. The challenge strain soon outnumbered the other *E. coli* types in the intestines of most piglets. Diarrhoea developed in the majority of piglets and its severity varied. Severe diarrhoea was observed in 10% of the piglets but only one piglet died due to dehydration. No inflammatory lesions were detected in the intestines of the dead piglet and the other euthanized piglets. We assume that development of haemorrhagic gastroenteritis depends on the involvement of other factors that need to be identified. *E. coli* O149:F4 are only one of the causative factors of haemorrhagic gastroenteritis in piglets after weaning.

*Haemorrhagic gastroenteritis, K88, diarrhoea, enterotoxins*

The postweaning *E. coli* diarrhoea (PWED) is caused by enterotoxigenic *E. coli* strains (ETEC). Enterotoxigenic *E. coli* isolated from weaned piglets possessing colonizing factors F4 (K88) and having the ability to produce enterotoxins usually belong to serogroup O149 (Tzipori 1985; Soderlind et al. 1988; Imberechts et al. 1994). Strains possessing colonizing factors F18 usually belong to different O-serogroups (Nagy et al. 1992; Salajka et al. 1992; Fairbrother et al. 1994). Colonizing factors (adhesins) are fimbrial antigens which enable adherence of ETEC to intestinal mucosa. The colonization is necessary for both mucosal adhesion and proliferation. The ETEC of the serogroup O149, isolated from piglets that had died of severe dehydration largely express F4ac fimbriae (Noamani et al. 2003) and are usually able to produce both heat-labile (LT) and heat-stable (STb) enterotoxins (Imberechts et al. 1994). Enterotoxins induce secretory diarrhoea, generally without inducing histological changes, although STb may cause shortening of villi (Rose et al. 1987). Heat-labile enterotoxin plays a key role in the pathogenesis of diarrhoeal *E. coli*-infections, whilst STb only slightly affects the course of the disease (Zhang et al. 2006; Erume et al. 2008).

The course of *E. coli* infections in weanings can range from peracute to chronic. In some cases, however, the disease can resemble dysentery or “intestinal haemorrhagic syndrome” (Svendsen 1974). When peracute and acute disease emerges, the shock syndrome can occur, including haemorrhages or even haemorrhagic enteritis in the intestines. Some authors use the terms haemorrhagic gastroenteritis or colibacillary shock. During this type of disease, LT and STb producing ETEC of serogroup O149:F4 are most often isolated (Svendsen et al. 1974; Faubert and Drolet 1992). The following histological lesions are found in piglets that died: neutrophil infiltration of lamina propria, micro-vascular fibrinous blood clots in mucous and submucous layers of the intestine and necrosis of villi.

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In the Czech Republic, O149:F4 of ETEC with colonization factors F4 is the most frequently isolated serogroup from piglets that suffer from or died due to PWECD (Salajka et al. 1992). We isolated an almost pure culture of haemolytic *E. coli* O149:F4 (producing LT and STb, in some cases together with STa) from the intestines of the majority of fatal cases of weaned piglets with severe inflammation of intestines. This was associated with strong hyperaemia and oedematous, hyperaemic or haemorrhagic mucosa. The signs of "haemorrhagic gastroenteritis" found by gross autopsy are not consistent with the common pathogenesis of the disease that is explained by the effect of enterotoxins. Based on the facts and findings outlined above, we tried to extend the knowledge of ETEC O149:F4 pathogenesis in the present study. The aim of our study was to induce haemorrhagic gastroenteritis in piglets experimentally infected with ETEC O149:F4 strains isolated from piglets affected by haemorrhagic gastroenteritis and compare the cytokine response of intestinal leukocytes in affected piglets with control.

### Materials and Methods

#### ETEC strains used for infection

Haemolytic strains of *E. coli* 11462 (O149:F4, LT+, STb+), 11501 (O149:F4, LT+, STb+), 10416 (O149:F4, LT+, STb+), isolated from the intestines of dead piglets showing typical signs of haemorrhagic gastroenteritis and strain 11040 (O8:F4, LT+, STb+) isolated from the intestines of piglets without apparent inflammation of the intestines were used for infection. The cultures of *E. coli* were grown in culture medium containing 12.5 g of acid casein hydrolysate, 12.5 g of enzymatic casein hydrolysate (Imuna, Šarišské Michalany, Slovak Republic) and 0.5 g of yeast extract (Oxoid) per 1 litre. The cultures were incubated at 37 °C with shaking for 16 h. A part of the cultures for individual oral administration were pelleted by centrifugation and mixed into a neutral sterile puree (wheat flour and semolina). The paste contained  $2 \times 10^{10}$  CFU/ml.

#### Animals

Animal handling complied with the legal directives of the Czech Republic and with the Institute's policy. Clinically healthy piglets from a conventional herd (53 animals in 3 experimental groups with 30, 12 and 11 animals and 8 animals in the control group), weaned at the age of 28 days were used in the experiments. The piglets were fed a commercial diet COS 1 composed of 35.9% of wheat, 30.0% of barley and 18.5% of soybean meal (19.2% N substances, 3.5% fat and 3.7% fibre). The groups of piglets were kept in separated rooms.

#### Challenge of piglets

The experimental groups of piglets were challenged with ETEC strains O149:F4 (30, 12 and 11 piglets challenged with strain 11501, 11462 and 10416, respectively). The control group of piglets was challenged with ETEC strain O8:F4. All piglets were orally inoculated with an ETEC culture in a dose of  $2 \times 10^{11}$  CFU on the day after weaning. The diet for piglets was supplemented with ETEC culture of the same dose next day. The clinical status of the piglets was regularly monitored for 3 weeks after infection. Rectal swabs were collected every day for bacteriological examination. Animals that died or were euthanized *ante finem* were examined by autopsy; their intestines, mesenteric lymph-nodes, livers, spleens and kidneys were examined by culture.

#### Microbiological examination

Rectal swabs from piglets or contents of jejunum and colon were diluted in PBS (pH 7.4) and spread on blood agar containing 5% lamb blood and on MacConkey agar and cultured in an incubator at 37 °C. After superficial burning and cutting, liver, spleen, kidney and mesenteric lymph nodes obtained from the euthanized piglets were smeared onto 5% lamb blood and on MacConkey agar. The percentages of haemolytic colonies were assessed and 10 haemolytic colonies of *E. coli* from each swab were examined. Haemolytic colonies were inoculated onto a nutrient broth (Imuna, Šarišské Michalany, Slovakia). After incubation at 37 °C for 16 h, intravital staining of cultures was performed by adding TTC (triphenyl-tetrazolium-chloride) for 1 h and heating to 100 °C, and then these were examined by agglutination with antisera to O149 (Salajka et al. 1992). Presence of the F4 fimbriae was confirmed by agglutination with antisera. The percentage of the strain administered and present in faeces was calculated according to the numbers of haemolytic O149 type colonies in diluted culture on blood agar.

### Results

All piglets infected with ETEC developed diarrhoea after 16 h post infection. Severe diarrhoea was observed in 2 piglets infected with ETEC O149:F4 and ETEC O8:F4, respectively. One and two piglets with diarrhoea from each infected group were euthanized

48 h and 72 h after infection, respectively. The signs of diarrhoea associated with mild dehydration were identified by autopsy. Even though the intestines were filled with watery material, neither hyperaemia nor any other sign of inflammation were apparent. In all groups the course of the disease was comparable. In the groups of piglets challenged with strain 11462 or 10416, severe diarrhoea was observed in 10% of piglets. One piglet suffering from diarrhoea from each group was euthanized 48 h after infection. One piglet died on day 5 after O149:F4 ETEC (11462) administration. Autopsy indicated dehydration in all piglets, but no inflammation of the intestines was seen. Diarrhoea occurred in a majority of the infected piglets; however, it was mild in most cases. All other piglets survived without therapy.

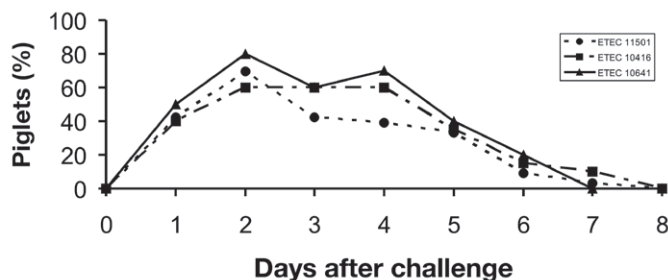


Fig. 1. Percentage of piglets with predominance of ETEC O149:F4 in faeces after challenge.

Enterotoxigenic *E. coli* strains used for the challenge of piglets were detected by bacteriological examination of rectal swabs on day 1, peaked on day 2 (Fig. 1) and decreased continuously until day 9 after administration. The ETEC predominated in faeces from a total of 42 piglets. In faeces of other piglets was part of ETEC lower than 50%. Investigation of the euthanized piglets and piglet that had died during experiment revealed that ETEC used for the challenge outnumbered the other *E. coli* strains in jejunum and colon of all but one piglet infected with ETEC O149:F4. The count of ETEC in the latter was lower than 50% of the total count of *E. coli*.

### Discussion

The aim of the present study was to induce “haemorrhagic gastroenteritis” which is often encountered at autopsy of weaning piglets. The ETEC of serogroups O138, O139, O141 and O149 are usually found in the intestines of piglets diagnosed with “haemorrhagic gastroenteritis”. However, serogroup O149 predominates in the majority of cases (Svendsen et al. 1974). In our experience from pig herds in the Czech Republic, O149:F4 ETEC were found in the intestines of almost all animal cases suffering from haemorrhagic inflammation. Before initiation of the experiment, we assumed that these ETEC types were the primary cause of haemorrhagic gastroenteritis. However, we did not observe any inflammation lesions in the intestines of piglets euthanized 48 and 72 h after infection and found no signs of enteritis in the piglet that had died spontaneously 5 days after infection ETEC. Nevertheless, bacteriological examination of the piglets showed sufficient colonization of the intestines with ETEC. Even though diarrhoea of varying intensities was observed in a majority of piglets, it was largely mild. In the study of Jensen et al. (2006), an infection was induced in piglets using the strain ETEC O149:F4ac and diarrhoea developed in most of them. Nevertheless, they failed to induce haemorrhagic gastroenteritis. In similar experiments, Sarmiento et al. (1988) induced diarrhoea with

the strain ETEC O157:F4 in 50% of the infected piglets and 3 out of 44 animals died. However, they only found diarrhoea and fluid distension of small and large intestines in dead piglets at autopsy. Jensen et al. (2006) infected F4ac/ab susceptible piglets after weaning with strains ETEC O149:F4 and described occurrence of diarrhoea in 74% of them. However, they did not mention any case of haemorrhagic gastroenteritis. In our experiment, only weak manifestation of the disease was observed, even though 3 different strains of O149:F4 isolated from haemorrhagic gastroenteritis cases were used.

It is known that genetic resistance to colonisation of the intestines with ETEC possessing colonization factors F4 exists (Rutter et al. 1975; Sellwood et al. 1975). Nevertheless, considering the number of infected piglets and the results of clinical and bacteriological examinations, we do not consider absence of F4 receptors in the piglets as the cause of the mild course of the disease without inflammation lesions in the intestines.

The finding of haemolytic *E. coli* strains in mesenteric lymph nodes is common in cases of serious enteric *E. coli* infections of piglets after weaning (Salajka and Salajkova 1987). They can also be occasionally isolated from the spleen (Svendsen et al. 1974). The isolates from cases of haemorrhagic gastroenteritis have often been diagnosed as O149:F4 (unpublished data). In the study of cytokine responses of intestinal epithelial cell line (IPI-2I) and macrophage cell line (3D4/31) after stimulation with different serotypes of ETEC (Pavlova et al. 2008) it was found that all the used serotypes were unable to induce IL-8 and TNF- $\alpha$  mRNA expression in IPI-2I cell line as measured by the real-time RT-PCR. However, in 3D4/31 cell line, differences in cytokine responses were detected among the used serotypes. The highest IL-8 and TNF- $\alpha$  mRNA expression in 3D4/31 was detected after stimulation with serotype O149:K88. Preconditions for ETEC O149:F4 crossing through the intestinal wall are unknown. However, having passed into the organism of piglets through the wall of the intestine, ETEC comes in contact with macrophages and can cause inflammation.

The onset of haemorrhagic gastroenteritis is characterized by a few sudden deaths of piglets in a litter (Svendsen et al. 1974). In accordance with other authors (Faubert and Drolet 1992), we have often observed diarrhoea in the herd, but not necessarily in piglets that suddenly died. Despite persistent diarrhoea, usually no further mortality occurs among piglets due to prompt antibiotic treatment. There is a question whether the therapy prevented further deaths of piglets due to haemorrhagic gastroenteritis or whether other infected piglets did not suffer from gastroenteritis but only from diarrhoea. This could explain why the experiment failed to actually induce cases of haemorrhagic gastroenteritis despite colonization of the intestine of piglets by ETEC. However, the underlying preconditions of ETEC passing through the intestinal wall and factors predisposing piglets to development of haemorrhagic gastroenteritis remain obscure. *E. coli* O149:F4 is only one of the causative factors of haemorrhagic gastroenteritis in piglets after weaning.

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